

09/832, 419

CLAIMS

What is claimed is:

1. A method for determining gene function between at least two genome-registered collections comprising:

5 (a) assembling at least two genome-wide scale, genome-registered collections;
(b) perturbing each collection from (a) with at least one perturbation;
(c) measuring the response of each collection to each perturbation of (b);
(d) analyzing the results of the at least one perturbation to identify patterns of similarities and differences between the at least two genome-registered collections.

10 2. A method according to Claim 1 wherein the perturbation is selected from the group consisting of radiation, humidity, alterations in temperature, alterations in carbon source, alterations in energy source, alterations in nitrogen source, alterations in phosphorus source, alterations in sulfur source, alterations in trace element sources, a change in pH, the presence other organisms, the presence of chemicals, the presence of toxins, and abnormal levels of normal metabolites.

15 3. A method for generating a genome-registered collection of reporter gene fusions comprising the steps of:

20 (a) generating a set of gene fusions comprising:
1) a reporter gene or reporter gene complex operably linked to
2) a genomic fragment from an organism of which at least 15% of the genomic nucleotide sequence is known;
(b) introducing *in vitro* the reporter gene fusions from step (a) into a host organism;
(c) registering the reporter gene fusions on the basis of sequence homology to the genomic sequence of the organism;
(d) repeating (a), (b), and/or (c) until reporter gene fusions have been made to at least 15% of the known genomic nucleotide sequence of said organism.

25 4. A method according to Claim 3 wherein the gene fusions of step (a) are generated either *in vivo* or *in vitro*.

30 5. A method for generating a genome-registered collection of reporter gene fusions comprising:

35 (a) generating random nucleic acid fragments from the DNA of an organism of which at least 15% of the nucleotide sequence is known;
(b) operably linking the random nucleic acid fragments generated in (a) to a vector containing a promoterless reporter gene or reporter gene complex;
(c) introducing the vector (b) containing the gene fusions into a host organism;

5 (d) determining the nucleic acid sequence of the distal and the proximal ends of the random nucleic acid fragments relative to the reporter gene or reporter gene complex;

10 (e) registering the sequenced fusions of step (d) on the basis of sequence homology to the genomic sequence of the host organism;

(d) repeating (a), (b), and/or (c) until reporter gene fusions have been made to at least 15% of the known genomic nucleotide sequence of said organism

15 6. A method according to Claim 5 wherein the random nucleic acid fragments of step (a) are generated by method selected from the group consisting of restriction enzyme digestion, physical shearing of the genome and polymerase chain reaction.

20 7. A method for generating a genome-registered collection of reporter gene fusions comprising:

25 (a) providing a genome from an organism wherein at least 15% of the nucleotide sequence is known;

30 (b) providing a series of amplification primers having homology to specific known regions of the genome of (a);

(c) amplifying portions of the genome of (a) with the primers of (b) to create a collection of nucleic acid amplification products;

(d) operably linking the amplification products of (c) to a vector containing a promoterless reporter gene or reporter gene complex;

(e) introducing the reporter gene fusions into a said organism;

(f) repeating (a) - (e) until, until reporter gene fusions have been made to at least 15% of the known genomic nucleotide sequence of said organism.

35 8. A method for generating a genome-registered collection of reporter gene fusions comprising steps of:

(a) introducing one or more transposons into the genome of an organism of which at least 15% of the nucleotide sequence is known, each transposon containing a promoterless reporter gene or reporter gene complex;

(b) determining the nucleic acid sequence of the junction between the proximal end of the genomic DNA and the transposon containing the reporter gene or reporter gene complex and registering the reporter gene fusions relative to the genomic sequence of the organism,

(c) repeating (a) and (b) until reporter gene fusions have been made to at least 15% of the known genomic nucleotide sequence of said organism

35 9. A method according to any one of Claims 1, 3, 5, 7 or 8 wherein organism is selected from the group consisting of prokaryotes and fungi.

10. A method according to Claim 9 wherein the prokaryote is an enteric bacterium.

5 11. A method according to Claim 10 wherein the enteric bacterium is selected from the group consisting of *Escherichia* and *Salmonella*.

10 12. A method according to one of Claims 1,3,5, 7 or 8 wherein the reporter gene or reporter gene complex is selected from the group consisting of *luxCDABE*, *lacZ*, *gfp*, *cat*, *galK*, *inaZ*, *luc*, *luxAB*, *bgaB*, *nptII*, *phoA*, *uidA* and *xylE*.

15 13. A method according to one of Claims 1, 3, 5, 7 or 8 wherein at least 50% of the genomic nucleotides sequence is known.

20 14. A method for identifying a profile of inducing conditions for a reporter gene fusion comprising:

25 (a) obtaining a gene expression profile of an organism under induced and non-induced conditions wherein induced genes are identified;

(b) providing a genome-registered collection of reporter gene fusions, said fusions registered to the genome of the organism of (a);

(c) selecting the reporter gene fusions of (b) that correspond to the induced genes of (a) to create a subset of the genome-register collection;

(d) contacting the subset of the genome-register collection of (c) with the inducing conditions of (a) to identify at least one representative reporter gene fusion whose expression was altered in a similar manner as in (a);

(e) contacting the at least one representative reporter gene fusion of (d) in a high throughput manner with a multiplicity of different inducing conditions to identify a profile of inducing conditions for that reporter gene fusion.

30 15. A method according to Claim 14 wherein at least 15% of the genomic nucleotide sequence of said organism is known.

35 16. A method for identifying a profile of inducing conditions for a reporter gene fusion comprising:

(a) obtaining a gene expression profile for each of mutant strain and a parental strain organism under induced and non-induced conditions wherein induced genes are identified;

(b) providing a genome-registered collection of reporter gene fusions, said fusions registered to the genome of the organism of (a);

(c) selecting the reporter gene fusions of (b) that correspond to the induced genes of (a) to create a subset of the genome-register collection;

(d) contacting the subset of the genome-register collection of (c) with the inducing conditions of (a) to identify at least one representative reporter gene fusion whose expression was altered in a similar manner as in (a);

(e) contacting the at least one representative reporter gene fusion of (d) in a high throughput manner with a multiplicity of different inducing conditions to identify a profile of inducing conditions for that reporter gene fusion.

17. A method to validate results from comprehensive genome analysis comprising the steps of:

- (a) analyzing a genome-wide, gene expression assay of an organism treated with a condition or chemical of interest to identify genes with altered expression;
- (b) selecting from a genome-registered collection of reporter gene fusions those reporter gene fusions containing promoter regions operably linked to genes corresponding to the altered genes from (a) or genes co-regulated with genes corresponding to the altered genes from (a);
- (c) testing expression of the reporter gene fusions selected from (b) with the conditions or chemicals of interest used in (a); and
- (d) comparing the gene expression results from (c) to the gene expression result of (a).

18. A method to determine operon structure comprising steps of:

- (a) selecting a subset of reporter gene fusions from a genome-registered collection of reporter gene fusions that map to the region of a possible operon;
- (b) assaying the subset for the reporter gene function; and
- (c) determining a putative operon structure based on the quantities of reporter gene function.

19. A method for constructing a cellular array containing reporter gene fusions comprising:

- (a) generating a set of gene fusions comprising:
 - 1) a reporter gene or reporter gene complex operably linked to
 - 2) a genomic fragment from an organism of which at least 15% of the genomic nucleotide sequence is known;
- (b) selecting a non-redundant subset of reporter gene fusions from the set of (a) representative of at least 15% of known or suspected promoter regions from a genome-registered collection of reporter gene fusions, each containing a known or suspected promoter region operably linked to a reporter gene or reporter gene complex; and
- (c) fixing the non-redundant subset of reporter gene fusions of (b) in an array format.

20. A method for measuring gene expression responses to perturbation comprising:

- (a) constructing at least 2 identical cellular arrays, each cellular array comprising a reporter gene fusion comprising:
 - 1) a reporter gene or reporter gene complex operably linked to

2) a genomic fragment from an organism of which at least 15% of the genomic nucleotide sequence is known;

wherein at least one cellular array is a control array and at least one cellular array is an experimental array;

5 (b) contacting the experimental array of (a) with a perturbing condition;
(c) comparing the differences between the gene expression activity of the control and the experimental array wherein gene expression response to a perturbing condition is determined.

21. The method of Claim 20 wherein the cellular array is fixed in a manner
10 selected from the group consisting of, fixed on a solid medium, and arrayed in liquid medium.

22. The method of Claim 20 wherein the perturbing condition is selected from the group consisting of radiation, humidity, alterations in temperature, alterations in carbon source, alterations in energy source, alterations in nitrogen source, alterations in phosphorus source, alterations in sulfur source, alterations in trace element sources, a change in pH, the presence other organisms, the presence of chemicals, the presence of toxins, and abnormal levels of normal metabolites.

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